

We claim:

1. A nanoscale nucleic acid sequence detection apparatus comprising:

a) a hydrophilic nonconductive substrate serving as a sample plate;

b) a cathode macroelectrode located on one surface of said substrate;

c) an anode macroelectrode located on said surface of said substrate such that the spacing between said cathode macroelectrode and said anode macroelectrode is greater than the length of one nucleic acid molecule, the spacing between said cathode macroelectrode and said anode macroelectrode defining a nucleic acid loading and delivery path;

d) a molecular transport liquid located on said surface of said substrate;

e) an injection device capable of introducing a sample nucleic acid molecule into said molecular transport liquid;

f) a programmable pulse generator connected to said cathode macroelectrode and said anode macroelectrode, said programmable pulse generator capable of controllably moving a nucleic acid molecule contained in said liquid along the nucleic acid loading and delivery path between said cathode macroelectrode and said anode macroelectrode by means of a programmable electrophoresis electric field;

g) a first nanoelectrode located on said surface of said substrate;

h) a second nanoelectrode located on said surface of said substrate such that the gap between said first nanoelectrode and said second nanoelectrode crosses the nucleic acid loading

and delivery path, the gap between said first nanoelectrode
and said second nanoelectrode defining a nanometer-size
nucleic acid detection gate on said hydrophilic nonconductive
substrate;

i) a first nonhydrophilic and nonconductive protective
insulating shield constructed on said surface of said
substrate along the sides of said first nanoelectrode, the
construction of said first protective insulating shield such
that only the tip of said first nanoelectrode remains exposed
on said surface of said substrate;

j) a second nonhydrophilic and nonconductive protective
insulating shield constructed on said surface of said
substrate along the sides of said second nanoelectrode, the
construction of said second protective insulating shield such
that only the tip of said second nanoelectrode remains exposed
on said surface of said substrate; and

k) a nucleic acid nucleotide base detection means located at
said nucleic acid detection gate.

2. The apparatus of claim 1, further comprising:

two parallel spaced-apart electrically conductive plates,
said plates arranged such that said sample plate is located
between said electrically conductive plates; and

a second programmable pulse generator connected to said
electrically conductive plates, said second programmable pulse
generator capable of applying a holding electric field across
said conductive plates in order to orient the nucleic acid
molecule contained in said liquid with respect to said sample
plate and said conductive plates.

3. The apparatus of claim 1 wherein said injection device is a micropipette, a microfluidic injection device, or a nanofluidic injection device.

4. The apparatus of claim 2 wherein the movement and orientation of a sample nucleic acid molecule is precisely controlled by coordinated action of said programmable electrophoresis electric field and said holding electric field.

5. The apparatus of claim 1 wherein the moving direction and step size of a sample nucleic acid molecule at said detection gate is controlled by adjusting the direction, amplitude, and duration of the programmable electrophoresis electric field.

6. The apparatus of claim 2 wherein a sample nucleic acid molecule is oriented with its negatively charged chain of phosphate groups pointing downward toward the surface of the sample plate, and its nucleotide bases pointing upward as desired for detection by using the holding electric field at the proper strength and in the correct direction, i.e., the electrically conductive plate beneath the sample plate positively charged.

7. The apparatus of claim 2 wherein a sample nucleic acid molecule is held at said detection gate for a period by a holding electric pulse from said second programmable pulse generator delivered through said parallel electrically

conductive plates so as to ensure reliable detection of the nucleotides.

8. The apparatus of claim 1 wherein the passage of a single nucleic acid molecule is achieved by use of detection gate spacing in the range of 1-10 nm.

9. The apparatus of claim 1 wherein the passage of a single nucleic acid molecule is achieved by use of detection gate spacing in the range of 2-6 nm.

10. The apparatus of claim 1 wherein the detection of a single nucleic acid molecule and reading of its nucleotide base sequence is achieved by use of a detection gate spacing in the range of 1-10 nm.

11. The apparatus of claim 1 wherein the detection of a single nucleic acid molecule and reading of its nucleotide base sequence is achieved by use of a detection gate spacing in the range of 2-6 nm.

12. The apparatus of claim 1 wherein said molecular transport liquid is provided and controlled by a relative humidity control system.

13. The apparatus of claim 1 further comprising a hydrophilic and nonconductive cover placed on the top sides of said macroelectrodes, said nanoelectrodes, and said protective shields to control the thickness of said molecular transport liquid on said hydrophilic nonconductive substrate.

14. The apparatus of claim 1 wherein said nucleic acid nucleotide base detection means is a tunneling current detector.

15. The apparatus of claim 1 wherein said nucleic acid nucleotide base detection means is a tunneling current spectroscope.

16. The apparatus of claim 1 wherein said nucleic acid nucleotide base detection means is a dielectric molecular detector.

17. The apparatus of claim 1 wherein said nucleic acid nucleotide base detection means is a high-resolution atomic force microscopic (AFM) probe.

18. The apparatus of claim 1 wherein said nucleic acid nucleotide base detection means is an electrostatic force microscopic (EFM) probe.

19. The apparatus of claim 1 wherein said molecular transport liquid is a chemical solution.

20. The apparatus of claim 1 wherein the passage of a single nucleic acid molecule and detection of its nucleotides is enhanced by use of appropriate solvent conditions such as pH and ionic strengths.

21. The apparatus of claim 2 wherein the actions of said nucleic acid nucleotide base detection and said electrophoresis and holding electric fields are coordinated and synchronized.

22. The apparatus of claim 1 is calibrated with standard nucleic acid samples of known sequences, and signal profiles of said sequences are established for each of the four distinct nucleotide bases: adenine (A), guanine (G), thymine (T)[uracil (U) if RNA], and cytosine (C).

23. The apparatus of claim 1 wherein the nucleotide sequence information of an unknown nucleic acid sample molecule is obtained by comparing its nucleotide base detection signals with the established signal profiles of the four distinct nucleotides with computer-assisted data fitting.

24. A nanoscale nucleic acid sequence detection apparatus comprising:

- a) a hydrophobic and nonconductive substrate serving as a sample plate;
- b) a cathode macroelectrode located on one surface of said substrate;
- c) an anode macroelectrode located on said surface of said substrate such that the spacing between said cathode macroelectrode and said anode macroelectrode is greater than the length of one nucleic acid molecule, the spacing between said cathode macroelectrode and said anode macroelectrode defining a nucleic acid loading and delivery path;

d) a first nanoelectrode located on said surface of said substrate;

15 e) a second nanoelectrode located on said surface of said
substrate such that the gap between said first nanoelectrode
18 and said second nanoelectrode crosses the nucleic acid loading
and delivery path, the gap between said first nanoelectrode
and said second nanoelectrode defining a nanometer-size
nucleic acid detection gate on said hydrophobic and
21 nonconductive substrate;

24 f) a hydrophilic sample loading and delivery area on said
hydrophobic and nonconductive substrate, said hydrophilic area
extending along said nucleic acid loading and delivery path
from said cathode macroelectrode to said anode macroelectrode,
said hydrophilic sample loading and delivery area constructed
27 so as to taper gradually less from said cathode macroelectrode
to said nucleic acid detection gate;

30 g) a molecular transport liquid located on said hydrophilic
sample loading and delivery area, said molecular transport
liquid preferentially tending to form a funnel-like liquid
delivery path on said hydrophilic sample loading and delivery
33 area;

h) an injection device capable of introducing a sample
nucleic acid molecule into said molecular transport liquid;

36 i) a nucleic acid nucleotide base detection means located at
said nucleic acid detection gate;

39 j) a first programmable pulse generator connected to said
cathode macroelectrode and to said anode macroelectrode, said
first programmable pulse generator capable of controllably
moving a nucleic acid molecule contained in said liquid along
42 the nucleic acid loading and delivery path between said

cathode macroelectrode and said anode macroelectrode by means of a programmable electrophoresis electric field;

45 k) two parallel spaced-apart electrically conductive plates,
said electrically conductive plates arranged such that said
sample plate is located between said electrically conductive
48 plates; and

51 l) a second programmable pulse generator connected to said
electrically conductive plates, said second programmable pulse
generator capable of applying a holding electric field across
said electrically conductive plates in order to orient the
nucleic acid molecule contained in said liquid with respect to
54 said sample plate and said electrically conductive plates.

25. The apparatus of claim 24 wherein said injection device
is a micropipette, a microfluidic injection device, or a
nanofluidic injection device.

26. The apparatus of claim 24 wherein the movement and
orientation of a sample nucleic acid molecule is precisely
controlled by coordinated action of said programmable
electrophoresis electric field and said holding electric
field.

27. The apparatus of claim 24 wherein the moving direction
and step size of a sample nucleic acid molecule at said
detection gate is controlled by adjusting the direction,
amplitude, and duration of the programmable electrophoresis
electric field.

28. The apparatus of claim 24 wherein a sample nucleic acid molecule is oriented with its negatively charged chain of phosphate groups pointing downward toward the surface of the sample plate, and its nucleotide bases pointing upward as desired for detection by using the holding electric field in the proper strength and in the correct direction, i.e., the electrically conductive plate beneath the sample plate positively charged.

29. The apparatus of claim 24 wherein a sample nucleic acid molecule is held at said detection gate for a period by a holding electric pulse from said second programmable pulse generator delivered through said parallel electrically conductive plates so as to ensure reliable detection of the nucleotides.

30. The apparatus of claim 24 wherein the passage of a single nucleic acid molecule is achieved by use of detection gate spacing in the range of 1-10 nm.

31. The apparatus of claim 24 wherein the passage of a single nucleic acid molecule is achieved by use of detection gate spacing in the range of 2-6 nm.

32. The apparatus of claim 24 wherein the detection of a single nucleic acid molecule and reading of its nucleotide base sequence is achieved by use of a detection gate spacing in the range of 1-10 nm.

33. The apparatus of claim 24 wherein the detection of a single nucleic acid molecule and reading of its nucleotide base sequence is achieved by use of a detection gate spacing in the range of 2-6 nm.

34. The apparatus of claim 24 wherein said molecular transport liquid is provided and controlled by a relative humidity control system.

35. The apparatus of claim 24 wherein said nucleic acid nucleotide base detection means is a tunneling current detector.

36. The apparatus of claim 24 wherein said nucleic acid nucleotide base detection means is a tunneling current spectroscope.

37. The apparatus of claim 24 wherein said nucleic acid nucleotide base detection means is a dielectric molecular detector.

38. The apparatus of claim 24 wherein said nucleic acid nucleotide base detection means is a high-resolution atomic force microscopic (AFM) probe.

39. The apparatus of claim 24 wherein said nucleic acid nucleotide base detection means is an electrostatic force microscopic (EFM) probe.

40. The apparatus of claim 24 wherein said molecular transport liquid is a chemical solution.

41. The apparatus of claim 24 wherein the passage of a single nucleic acid molecule and detection of its nucleotides is enhanced by use of appropriate solvent conditions such as pH and ionic strengths.

42. The apparatus of claim 24 wherein the actions of said nucleic acid nucleotide base detection and said electrophoresis and holding electric fields are coordinated and synchronized.

43. The apparatus of claim 24 is calibrated with standard nucleic acid samples of known sequences, and signal profiles of said sequences are established for each of the four distinct nucleotide bases: adenine (A), guanine (G), thymine (T)[uracil (U) if RNA], and cytosine (C).

44. The apparatus of claim 24 wherein the nucleotide sequence information of an unknown nucleic acid sample molecule is obtained by comparing its nucleotide base detection signals with the established signal profiles of the four distinct nucleotides with computer-assisted data fitting.